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Rumen Ciliate Protozoal Faunae of Bali Cattle (*Bos javanicus domesticus*) and Water Buffalo (*Bubalus bubalis*) in Indonesia, with the Description of a New Species, *Entodinium javanicum* sp. nov.

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ABSTRACT — Rumen ciliate compositions were surveyed on the Bali cattle and the water buffaloes in Indonesia. As the result of survey, 15 genera including 45 species with 15 formae and 11 genera including 37 species with 12 formae were identified from the Bali cattle and the water buffalo, respectively. Of the ciliates detected from the Bali cattle, one new entodiniid species was recognized, then described as *Entodinium javanicum* sp. nov. The ciliate compositions between these two species of hosts were similar to each other, or 30 species detected were common. From the comparison of these faunae with that of the domestic animals fed in neighbouring area, the presence of geographical distribution of the rumen ciliates was strongly indicated. Some ciliate species detected only from the animals of Southeast Asia were discussed on the phylogenetical relationship to the host species. The average density of ciliates per 1 ml of the rumen fluid was 7.8×10^4 in the Bali cattle and 1.5×10^4 in the water buffaloes, respectively. The ciliates of the genus *Entodinium* was the most predominant both in the Bali cattle and the water buffaloes.

INTRODUCTION

The ciliates inhabiting the rumen of ruminants have been recognized as one of the highly evolved groups of ciliates, which have no related free-living species, and most of them possess peculiar morphological aspects [1]. Since the transfaunation of them has been assumed to be performed only due to the direct contact between the hosts [2], it is considered that rumen ciliate faunae have peculiarly developed in the rumen as narrow and limited habitat of host animals and would be different among the different species of hosts and/or among the hosts inhabiting different areas. Thus, the investigations on the rumen ciliate faunae of various ruminants in different areas, and the comparison of the results seem to offer the useful informations for the discussion on the phylogenetic relations not only among the rumen ciliates but also among the host ruminants.

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From this point of view, we have surveyed the rumen ciliate faunae of ruminants in various areas, especially around Southeast Asia, which have been still left out of investigation, and already reported on the microfaunae of the domestic [3, 4] and wild [5] ruminants in Japan, and of water buffalo [6–8] and zebu cattle [7, 8] in several countries of Southeast Asia.

The present paper deals with the ciliate faunae of the Bali cattle and of the water buffalo in Indonesia as one of these studies. One new species found from the Bali cattle in this examination is also described.

MATERIALS AND METHODS

Rumen contents examined were collected from 8 Bali cattle and a water buffalo slaughtered in the abattoir in Tanjung-Karang, Ranpong, and 16 water buffaloes slaughtered in Bogol, Jawa Island, Indonesia in January, 1983. All the hosts had been fed continuously on the true grasses without concentrates. Those samples were immedi-

ately fixed in twice volume of MFS (methylgreen-formalin-saline) solution [1] and brought to the laboratory. Then, the samples were added further with twice volume of MFS solution and stocked at darkened place until examination. Identification and calculation of ciliates in the stocked samples were examined light microscopically. Identification of genera and species and terminology of the ciliates were mainly in accordance with the descriptions published by Ogimoto and Imai [1]. The ciliates not described in their description were identified in conformity to various investigators [9–13]. The orientation of ciliates for description was in accordance with Ogimoto and Imai [1]; that is, the side closest to the macronucleus was called the "right side" in Entodiniinae and the "left side" in Diplodiniinae and Ophryoscolecinae, with the opposite side "left" and "right", respectively, and the other sides were called the "upper" and "lower" side. The total ciliate number was calculated from the number in 0.1 ml of the sample with a plankton counter deck glass (Rigosha, Tokyo) on which the lines were cut at 0.5 mm intervals, and converted into logarithms per 1 ml of the rumen fluid. The generic composition was shown as the percentage of each genus occupying in about 500 individuals.

A part of the samples of the Bali cattle, in which the new species was recognized, was stained with Mayer's hematoxylin staining and prepared as permanent slides for type specimens.

RESULTS

1. Description of a new species

On the course of survey, one new ciliate was recognized from the samples of 3 Bali cattle. The morphological characters of the new species is as follows;

Entodinium javanicum sp. nov.

(Figs. 1 and 2)

Diagnosis: Body elliptical, 29–32×19–21 μm: a length to width ratio 1.52; with two heavy caudal spines, the right spine being shorter than the left one; macronucleus thick and short at the anterior right side of body.

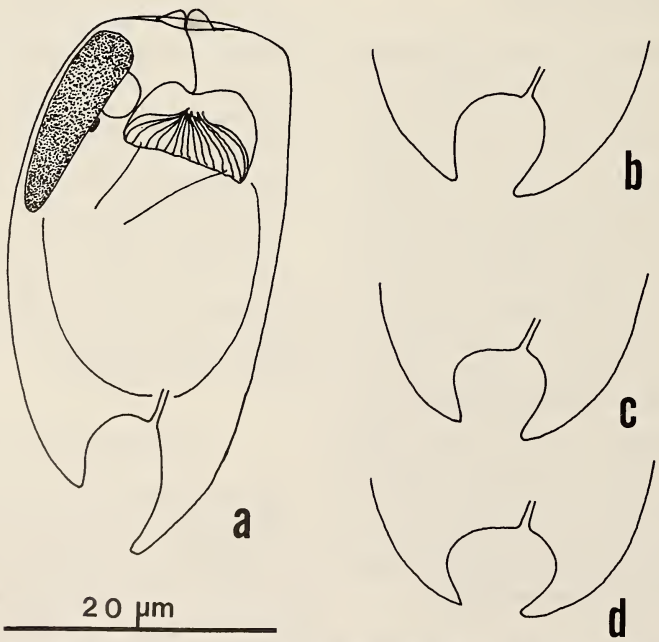

Fig. 1. *Entodinium javanicum* sp. nov. a: Lower view of whole body. b–d: Variation of the caudal spines.

FIG. 2. Light micrographs of *Entodinium javanicum* sp. nov. a: Lower view of a specimen fixed and stained with MFS solution. ×550. b: Upper view of a specimen stained with Mayer's hematoxylin. ×550.

Description: Viewed from the upper or lower side, the body is elliptical, but right surface of body is more convex than the left surface. The anterior end of body is relatively flattened when the adoral cilia are retracted. The rectum is apparent and situated at the slightly left side of posterior extremity of body and curve somewhat inwards. The right spine is shorter than the left one. These spines, especially left one, often show some variation in size (Fig. 1b–1d). The esophagus is funnel-shaped and bent rightward. A thick and short macronucleus is situated in the antero-lateral part of body, which is about one-third of the body length. The anterior end of the macronucleus is thicker than the posterior end. An ovoidal micronucleus is situated near the middle of the left margin of the macronucleus. A contractile vacuole lies close to the upper left part of anterior one-fifth of the macronucleus, just anterior to the micronucleus.

Measurement of 20 individuals of this species is as follows. In the description of body length, the size of caudal spine is not contained.

	Mean	SD	Range
Length (μm)	29.9	1.3	29–32
Width (μm)	19.7	0.6	19–21
Caudal spine (μm)	5.2	1.1	4– 7
L/W	1.52	0.04	1.5–1.6

Type host and locality: Bali cattle, *Bos javanicus domesticus*, in Ranpong, Indonesia.

Habitat: Rumen.

Occurrence: *E. javanicum* was detected in 37.5% of the Bali cattle surveyed.

Specimens deposited: Holotype and 2 paratypes on slides. They are deposited in the Department of Parasitology, Nippon Veterinary and Zootechnical College, Musashino, Tokyo, Japan.

Remarks: *Entodinium javanicum* is closely related to *Entodinium fujitai* Imai, 1981 [14] which has been detected from the tropical ruminants in Southeast Asia in the situation of the macronucleus and in the presence of two caudal spines. It is, however, distinguished from *E. fujitai* by the following points: (1) the left surface of body being not concave, (2) slender body (L/W; $t=8.84$, $n=20$, $P<0.001$), (3) slight difference of the situation of contractile vacuole, and (4) the left spine always larger than the right one and not so sharply as to be surrounded by right one.

Comment: Other than *E. fujitai*, *E. rostratum* [9], *E. ciculum* [13] and *E. parvum monospinosum* [15] are also similar to the present species in having one or two caudal spines. However, these species have long macronuclei which extend over a half of the body length, and they are able to be easily distinguished from *E. javanicum* and *E. fujitai* in this difference.

For the identification of rumen entodiniomorphid ciliates, the caudal spine is one of the most attractive features. Also in the present case, the shape of caudal spines is the most distinct character to identify *E. javanicum* from other related species. However, it has been indicated [5, 9] that the caudal spines of rumen ciliates have often wide variations in a species, so that they are not always suitable as a diagnosis of species. From this reason, it still remains a possibility that *E. javanicum* should be classified as one forma of *E. fujitai*. But every individual of *E. fujitai* detected up to this time from several species of the hosts involving the water buffalo in this examination [6–8] showed the identical morphology as the original description [14]. In contrast, neither *E. fujitai* nor confusing individuals for identification were detected from the Bali cattle. From this fact in addition to the

Table 1. Species of rumen ciliate protozoa found from the Bali cattle and water buffalo in Indonesia and their frequency of appearance

Family	Species	Frequency of appearance (%)	
		Bali cattle (n=8)	Water buffalo (n=17)
Isotrichidae	*Dasytricha ruminantium*	100.0	23.5
	Oligoisotricha bubali	37.5	41.2
	Isotricha intestinalis	62.5	—*
	prostoma	50.0	—
Blepharocorythidae	*Charonina ventriculi*	25.0	29.4
Ophryoscolecidae	*Entodinium*		
Entodiniinae	*simplex*	100.0	82.4
	longinucleatum		
	f. *longinucleatum*	100.0	47.1
	f. *actonucleatum*	37.5	23.5
	f. *spinolobum*	37.5	—
	ovinum	75.0	70.6
	exiguum	50.0	64.7
	minimum	75.0	—
	furca	50.0	5.9
	parvum		
	f. *parvum*	37.5	52.9
	f. *monospinosum*	50.0	35.3
	caudatum		
	f. *caudatum*	37.5	17.6
	f. *lobosospinosum*	50.0	17.6
	rostratum	37.5	58.8
	nanellum	37.5	29.4
	javanicum	37.5	—
	aculeatum	37.5	—
	chatterjeei	25.0	64.7
	dubardi	25.0	29.4
	indicum	25.0	23.5
	bimastus	25.0	17.6
	bifidum	25.0	5.9
	tsunodai	12.5	—
	bubalum	12.5	—
	costatum	12.5	—
	biconcavum	—	11.8
	fujitai	—	11.8
Diplodiniinae	*Diplodinium anisacanthum*		
	f. *anacanthum*	37.5	76.5

Family	Species	Frequency of appearance (%)	
		Bali cattle (n=8)	Water buffalo (n=17)
	f. *monacanthum*	25.0	17.6
	f. *diacanthum*	12.5	41.2
	f. *anisacanthum*	50.0	—
	f. *pentacanthum*	37.5	—
	f. *triacanthum*	—	23.5
	polygonale	75.0	41.2
	minor	—	17.6
	Eodinium		
	rectangulatum	50.0	11.8
	posterovesiculatum	12.5	52.9
	Eremoplastron		
	rostratum	75.0	58.8
	bovis	12.5	11.8
	bubalus	62.5	29.4
	dilobum	62.5	—
	monolobum	12.5	—
	Eudiplodinium		
	maggii	87.5	70.6
	Elytroplastron		
	bubali	50.0	—
	Diploplastron		
	affine	37.5	—
	Metadinium		
	medium	87.5	11.8
	ypsilon	25.0	11.8
	Ostracodinium		
	obtusum	50.0	17.6
	nucleolobum	37.5	11.8
	mammosum	25.0	17.6
	gracile	12.5	23.5
	trivesiculatum	—	35.3
	tiete	—	29.4
	Enoploplastron		
	triloricatum	37.5	—
Ophryoscolecinae	*Epidinium*		
	ecaudatum		
	f. *caudatum*	62.5	23.5
	f. *parvicaudatum*	37.5	—
	f. *quadricaudatum*	12.5	—
	f. *ecaudatum*	—	17.6
Total genera, species and formae		15 genera 45 species 15 formae	11 genera 37 species 12 formae

* Not detected.

morphological differences mentioned above, *E. javanicum* was decided to be a separate species from *E. fujitai*.

2. Composition of rumen ciliates

Table 1 shows genera, species and their incidences (No. of the hosts in which the species was detected/No. of examined hosts) of the ciliates found from the Bali cattle and the water buffalo in this examination. Those were 15 genera containing 50 species with 17 formae. Of them, 15 genera containing 45 species with 15 formae were recognized from the Bali cattle, and 11 genera containing 37 species with 12 formae from the water buffalo. Though most of them were common between both hosts, 13 species and 5 formae of 3 species were not detected from the water buffalo but from the Bali cattle, and 5 species and 2 formae of 2 species had the reverse relation.

Of the ciliate species found from the Bali cattle, *Dasytricha ruminantium*, *Entodinium simplex* and *E. longinucleatum longinucleatum* were the most popular, each incidence of which was 100.0%. In addition, *Isotricha intestinalis*, *I. prostoma*, *Entodinium ovinum*, *E. exiguum*, *E. minimum*, *E. furca*, *E. parvum monospinosum*, *E. caudatum lobosospinosum*, *Diplodinium anisacanthum anisacanthum*, *D. polygonale*, *Eodinium rectangulatum*, *Eremoplastron rostratum*, *Er. bubalus*, *Er. dilobum*, *Eudiplodinium maggii*, *Elytroplastron bubali*, *Metadinium medium*, *Ostracodinium obtusum* and *Epidinium ecaudatum caudatum* were detected from over a half number of the hosts examined.

On the other hand, there were no species detected from all the water buffalo, but *E. simplex* was the most predominant species of which the incidence was 82.4% in the animals examined. Over a half of the water buffaloes had *E. parvum parvum*, *E. rostratum*, *E. chatterjeei*, *D. anisacanthum anacanthum* and *Eo. posterovesiculatum* which showed relatively low incidence in the Bali cattle, in addition to *E. ovinum*, *E. exiguum*, *Er. rostratum* and *Eu. maggii* which had high incidence also in the Bali cattle.

Table 2 shows the percentage compositions of genera detected in this examination. Both in the Bali cattle and the water buffalo, the percentage occupied by the ciliates of *Entodinium* was the highest, the ratio of which was 67.8% and 74.6% on average, respectively. In the water buffalo, the ciliates of *Diplodinium* occupied next high composition, which value was 10.5%, then

Table 2. Generic composition (%) of the rumen ciliate protozoa in the Bali cattle and water buffalo in Indonesia

Genus	Bali cattle		Water buffalo	
	Mean	Range	Mean	Range
Dasytricha	7.2	0.5–23.5	0.5	0 – 3.5
Isotricha	1.4	0 – 3.5	0	—
Oligoisotricha	0.4	0 – 1.5	2.2	0 –20.0
Charonina	0.6	0 – 3.0	1.1	0 – 9.0
Entodinium	67.8	51.5–84.0	74.6	54.5–93.0
Diplodinium	6.1	0 –19.5	10.5	1.0–26.5
Eodinium	1.5	0 – 6.0	1.7	0 – 6.0
Eremoplastron	4.2	1.0–18.0	2.8	0 – 7.5
Eudiplodinium	3.5	0 – 7.5	1.6	0 – 4.0
Diploplastron	0.9	0 – 4.0	0	—
Metadinium	2.1	0 – 5.0	0.1	0 – 2.0
Ostracodinium	1.5	0 – 5.0	4.1	0 –12.5
Elytroplastron	0.6	0 – 2.0	0	—
Enoploplastron	0.5	0 – 2.0	0	—
Epidinium	1.5	0 – 3.5	0.9	0 – 4.5

Table 3. Average number of species appeared and average ciliate density in the Bali cattle and water buffalo in Indonesia and in the domestic ruminants in other areas

Host	Locality	Number of species			Ciliate density (log_{10}/ml)		
		Mean	SD	Range	Mean	SD	Range
Bali cattle (n=8)	Indonesia	21.5	4.8	14–30	4.89	0.24	4.6–5.4
Water buffalo (n=17)	Indonesia	12.9	3.7	8–20	4.19	0.77	2.8–5.5
Zebu cattle (n=46)	Thailand[1]	26.1	5.8	14–39	4.85	0.45	3.8–5.5
Zebu cattle (n=4)	Philippines[2]	16.5	5.0	11–22	4.97	0.26	4.7–5.4
Water buffalo (n=10)	Thailand[1]	9.4	4.5	2–17	3.87	0.30	3.2–4.3
Water buffalo (n=29)	Taiwan[3]	11.5	6.5	3–25	4.95	0.88	3.7–6.5
Cattle (n=69)	Japan[4]	10.3	4.0	4–25	5.13	0.61	3.7–7.6

Cited from [1] Imai and Ogimoto [8], [2] Shimizu *et al.* [7], [3] Imai *et al.*[6], and [4] Imai *et al.* [4].

of *Ostracodinium* was 4.1%, and of other genera were less than 3%. In the Bali cattle, the composition ratios had a tendency to be more balanced compared with that in the water buffalo, and the ratios of *Dasytricha*, *Diplodinium*, *Eremoplastron* and *Eudiplodinium* were more than 3%.

The average number of species per head of host and the total ciliate density per milliliter of rumen fluid of the domestic ruminants examined, and those of the animals fed in neighbouring area which had been reported by us [4, 6–8] are shown in Table 3. The average number of ciliate species in the Bali cattle was considerably higher than in the water buffalo; that is, 21.5 in the Bali cattle and 12.9 in the water buffalo, respectively. The average total ciliate number was 7.8×10^4/ml in the Bali cattle and 1.5×10^4/ml in the water buffalo.

DISCUSSION

Water buffaloes are the most popular domestic ruminants around the tropical area with zebu cattle. Those are classified to two types, river type and swamp type. In Southeast Asia, the latter type has been widely distributed. The rumen ciliate compositions of these animals have been reported on the swamp type ones in Taiwan [6], Philippines [7], Thailand [8] and Brazil [13], and on the river type ones in India [16] and Kafkas [17]. All of the species detected from the water buffalo in this examination had been found from any of those areas. Of these areas, however, the ciliate composition in this examination was the most similar to that in Thailand; that is, 21 of 26 species (80.8%) found from the water buffalo in Thailand were common with the species detected from Indonesia. Thirty of 42 species (71.4%) from Taiwan were also common. In contrast, the lowest common rate was obtained when the ciliate composition was compared with that of Brazil, or only 21 of 43 species (48.8%) was common. The common ratios in the ciliate compositions of the water buffaloes in India and Kafkas to that in Indonesia were 50.0% (10 of 20 species) and 67.9% (19 of 28 species), respectively. In the water buffalo in Philippines, though only 10 species were described from 10 animals, all of them were detected in this examination.

On the other hand, Bali cattle were domesticated from wild Banteng (*Bos javanicus*) inhabiting the limited area of Southeast Asia, and up to this time they are only kept at a part of Indonesia. They have been recognized to develop without cross to other wild or domestic cattle [18]. In general, zebu cattle (*Bos taurus indicus*) have widely been kept as domestic ruminants with water buffaloes around the tropical area, and their rumen ciliate compositions have been reported on those in Philippines [7], Thailand [8], India [10–12], China [19] and Senegal [20]. All of the ciliate species detected from the Bali cattle have been also detected from the zebu cattle in somewhere except *E. javanicum*, *E.*

costatum and *Epidinium ecaudatum parvicaudatum*. Especially, the ciliate composition of Bali cattle was resembled that of zebu cattle in Thailand like the case of the water buffalo; that is, 40 of 53 species (75.5%) detected from the Thailander cattle were also detected from the Bali cattle. On the other hand, 25 of 46 species (54.3%) from Chinese zebu cattle, 20 of 51 species (39.2%) from Indian zebu cattle and 18 of 33 species (54.5%) from Senegalese zebu cattle were common with that from Indonesian Bali cattle.

The ciliate compositions in the water buffalo and in the Bali cattle in Indonesia were very similar to each other, and 30 of 37 species from the water buffalo and of 45 species from the Bali cattle were common although slight difference was noticed on the predominant species in the animals.

These results that most species from the water buffalo in Indonesia were common with the ciliates from the Bali cattle fed in same area, that the ciliate compositions of Bali cattle and Thailander zebu cattle were similar to each other, and that even among the water buffaloes, common rate of ciliate species appearance was high in the animals in Indonesia and Thailand but low in Indonesia and in Brazil or Kafkas, will strongly indicate the presence of geographical distribution of the rumen ciliates.

Since the transfaunation of rumen ciliates to other hosts has been assumed to be performed orally [2], it is considered that ciliates are hardly transmitted if the hosts, even though they are the same species, are kept in far distance; in contrast, the transfaunation would easily occur among the hosts kept in narrow area even these hosts are different species.

Entodinium simplex, *E. longinucleatum longinucleatum*, *E. ovinum*, *E. exiguum*, *Eremoplastron rostratum* and *Eudiplodinium maggii* which were detected with high incidence both from the water buffalo and the Bali cattle in this examination have hitherto been ordinarily reported not only from tropical ruminants but also from cattle (*Bos taurus taurus*), sheep (*Ovis aries*) and goat (*Capra hircus*) [3, 9]. This fact suggests that the ciliates with high occurence have wide distribution range because of having many chances for transmission to other hosts. In contrast, the ciliates with low incidence would play as a factor to show the phylogenetical and/or geographical distribution of ciliate fauna because those have few chances for transmission so that their distribution is limited to narrow area. Of the species which had low incidence in this examination, *E. longinucleatum spinolobum*, *E. parvum monospinosum*, *E. tsunodai*, *E. bubalum* and *E. fujitai* in addition to *E. javanicum* newly described have been reported only from the ruminants in Southeast Asia. As mentioned above, it would be possible to consider that low incidences resulted in their limited distribution. In this point of view, it is suggested a possibility that the origin of these species may present in the ruminants in this area. Namikawa [21] reported that the domestic ruminants in Southeast Asia are fairly affected from the Banteng which is an ancestor type of Bali cattle on the basis of the comparison of distribution of hemoglobin-beta alleles in those animals. Consequently, the origin of some of the species mentioned above may be resulted in Banteng.

The percentage composition was the highest in the genus *Entodinium* both in the water buffalo and the Bali cattle surveyed in this examination, or about 75% and 68% of the total ciliates, respectively. The result would be strongly related to the high incidence and the wide distribution of this genus. Imai *et al.* [4] have reported from the survey of 69 cattle in Japan that the entodiniid ciliates occupied 86.5% of the total ciliates in the Japanese cattle, the percentage composition of other genera were less than 4% each. In this examination, the percentage composition of Diplodiniinae including the genus *Diplodinium* was higher than in the Japanese cattle. Since it is reported that entodiniid ciliates lack the ability of cellulose digestion, which the ciliates of Diplodiniinae have [22], it is considered to be reasonable that the tropical ruminants which are generally kept only by roughage have such rumen ciliate compositions.

The average number of species appeared per head of the host was apparently higher in the Bali cattle than in the water buffalo. As shown in Table 3, water buffaloes have generally poorer ciliate composition than in other domestic animals

such as cattle and zebu cattle. The result obtained in this examination would support this tendency. Though it is still not clear by what this difference is caused, the difference of feeding manner including the kinds of food for the hosts is considered to be one of the factors. On the other hand, the average ciliate number in the water buffalo and the Bali cattle in Indonesia was 1.5×10^4/ml and 7.8×10^4/ml, respectively, which values were apparently lower than in the Japanese cattle in which the value was 1.3×10^5/ml on average [4]. Such low values have also been noticed in the water buffalo and the zebu cattle in the areas other than Indonesia. It is reported that the ciliate density in the rumen is closely related to the kinds of food taken by the host [23], so that such feeding conditions of the domestic ruminants in tropical area appear to be not so good for the rumen ciliates. The decrease of ciliate number would cause the poor ciliate composition in each host. When the ciliates are considered as a protein source for the host [22], the low level of ciliate number may have disadvantageous influence nutritionally on such tropical ruminants. However, it is indicated that the Bali cattle have a varied composition of the rumen ciliates since the average species number appeared was high in spite of the low number of ciliates. It may indicate that this variety is due to many chances of the experiences to contact with other ruminants around neighbouring area.

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